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IDENTIFICATION OF GENES UNDERLYING THE QTL REGIONS IN THE QTL-NILS OF IR64/AZUCENA

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ABSTRACT

Rice, an important cereal crop of the world is constrained by a variety of factors in exhibiting its full yield potential, especially in the rainfed growing regions. One of the important factors limiting its growth is drought, to mitigate which, several strategies that efficiently employ a gamut of QTL that control root morphology has been identified. In the present study, 4 such QTL from a NIL population of IR64/Azucena have been identified. The genes that could reside in these regions have been analysed through ePCR as well as through BLAST. Several house keeping genes as well as those that directly contribute to drought tolerance and or resistance have been identified.

KEYWORDS: QTL from a NIL, IR64/Azucena, BLAST

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INTRODUCTION

As one of the world's most important cereal, rice (*Oryza sativa* L.) demands a productive growth environment. However, with the rampant and imminent climate change coupled with a horde of constraints that limit water availability (Krishna and Hittalmani, 2009, Naresh Babu, 2011, Keshavamurthy, 2011) in the rice growing belts, it becomes imperative to breed varieties that perform consistently under stress and under non-traditional aerobic environments, especially since rice suffers heavily during its critical reproductive growth stage (Lanceras *et. al*., 2002, Lafitte *et. al*, 2003). The manipulation of root systems is a well proven strategy in the development of tolerant genotypes towards drought (Yoshida and Hasegawa, 1982; Ekanayake *et. al*., 1989; O'Toole and Bland, 1987; Thanh *et. al*., 1999, Venuprasad *et. al*., 2012), with several QTL regions being identified across breeding material (Price and Tomos, 1997; Yadav *et. al*., 1997; Courtois *et. al*., 2000; Kamoshita *et. al*., 2002, Vaishali, 2003) in rice.

Identification of the genes underlying QTLs is yet another aspect of identifying useful combinations of QTLs in breeding exercises. Given the abundant QTLs that have been identified across species and traits, identifying genes present in the QTL regions offers precision in analyzing and understanding its effects. Genes underlying QTLs for abiotic stress resistance/ tolerance such as cold stress (Rabbani *et. al*. 2003), salt stress (Oztur *et. al*., 2002; Rabbani *et. al*., 2003) and drought stress (Garg *et. al*., 2002; Malatasri *et. al*., 2002; Agarwal *et. al*., 2002; Wang *et. al*., 2005,) have been elucidated.

MATERIALS AND METHODS

Plant Material

A hundred and thirty-five lines of a doubled haploid (DHLs) population derived from a cross between IR64, a high yielding, lowland, *indica* variety and Azucena, a traditional upland, aromatic *japonica* variety was developed by Guiderdoni *et. al.* (1988) at IRRI. From out of these lines, Shen *et. al.* (2001) developed a set of twenty nine near isogenic lines (NILs) based on the molecular information developed by Yadav *et. al.* (1997) on root morphology. Twenty-nine near-isogenic lines of IR64 (*indica*, high yielding) with QTL introgressions from Azucena (*japonica*, drought tolerant) controlling root morphology (QTL Introgressed Lines (QILs)) developed by Shen *et. al.* (2001) and fine mapped by Vaishali (2003) (Table 1), was used for the study.

IDENTIFICATION OF GENES PRESENT IN THE QTL REGIONS

Electronic Polymerase Chain Reaction' approach ('ePCR'; Schuler, 1997) using Perl regular expression alignment of primer sequences with orientation and threshold distance constraints to identify putative PCR amplicons in target sequences was used to arrive at the genes that reside in each of the QTL region. The primers for analysis were obtained from the Gramene database (Ware *et. al.*, 2002; **www.gramene.org**). Markers were correlated with the physical map by ePCR, run against the rice genome BAC sequences, retrieved from the Gramene database and by BLAST (Altschul *et. al.*, 1990) alignment searches of the rice BAC sequences.

The genes were also identified by searching in the QTL2gene facility developed by the Zhejiang University of Science, China (Wang *et. al.*, 2005; **http://ibi.zju.edu.cn.qtl2gene/qtl2gene.htm**). The genes were also identified after downloading the information between the flanking markers found in Gen Bank (**www.ncbi.nlm.nih.gov**) and from the rice databases of The Institute for Genomic Research (TIGR; **www.tigr.org**). These annotations were assessed using the *gbrowse* sequence browser from the Genetic Model Organism Database project (http://www.gmod.org; Stein *et. al.*, 2002; Lewis *et. al.*, 2002).

RESULTS AND DISCUSSIONS

BLAST algorithm is a basic search engine that aligns query sequences against sequences stored in databases based on certain conserved/ consensus domains. In the present investigation, the sequences between the flanking markers on each of the four chromosomes: 1, 2, 7 and 9 were obtained from the Rice Genome Sequence that is available in the public domain. The sequence was then subjected to BLAST analysis and the results are presented in Table 2. The relative proportion of the identified genes have been depicted in Plate 1. Several house-keeping genes have been identified at all four chromosomal regions, in addition to biotic stress resistance genes. Among the genes that contribute to drought tolerance and or resistance, alpha-trehalose phosphate synthase (UDP-forming) 123K chain gene, a fragment of subtilisin like protease, heat shock protein 70 and cytosolic fructose-bisphosphate aldolase were identified on chromosome1. 14-3-3 like protein, extension-like protein, gamma - Tip protein, dnaj protein homolog 1 and thioredoxin reductase (NADPH) were identified on chromosome 2. On chromosome 7, glutathione S-transferase and calcium dependant protein kinase were identified, while on chromosome 9, POS18 protein were identified. None of these stress specific genes showed overlap between chromosomal regions.

CONCLUSIONS

Results indicated the presence of several housekeeping genes that function in cell maintenance and metabolism. Specific genes for stress resistance were found on chromosome 1, 2 and 7. None of the genes identified from the sequence obtained from chromosome 9 were related to drought resistance.

REFERENCES

1. *Agrawal, G.K., Rakwal, R. and Iwahashi, H., 2002, Isolation of novel rice (Oryza sativa L.) multiple stress responsive map kinase gene, osmsrmk2, whose mRNA accumulates rapidly in response to environmental cues. Biochem. Biophys. Res. Commun., 294(5):1009-16.*
2. *Courtois, B., Mclaren, G., Singh, P.K., Yadav, R., Shen, L., 2000, Mapping QTLs associated with drought avoidance in upland rice. Mol. Breed., **6**: 56-66.*
3. *Ekanayake, I.J., De Datta. and Steponkus., 1989, Spikelet sterility and flowering response of rice to water stress at anthesis. Ann. Bot., **63**: 257-264.*
4. *Garg, A.K., Kim, J.K., Owens, T.G., Ranwala, A.P., Choi, Y.D., Kochian, L.V. and Wu, R.J., 2002, Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses. Proc. Natl. Acad. Sci. U.S.A., 99(25):15898-15903.*
5. *Guiderdoni, E.J., Glaszmann, J.C. and Courtois, B., 1988, Segregation of 12 isozyme genes among doubled haploid lines derived from a japonica x indica cross of rice (Oryza sativa L.). Euphytica 42: 45-53.*
6. ***http://ibi.zju.edu.cn.qtl2gene/qtl2gene.htm***
7. *http://www.gmod.org*
8. *Kamoshita, A., Zang, J., Siopongco, J., Salarimg, S., Nguyen, H. T. and Wade, L. J., 2002, Effect of Phenotyping environment on identification of quantitative trait loci for rice root morphology under anaerobic condition. Crop Sci., **42**: 255-265.*
9. *Keshava Murthy B. C., Arvind Kumar and Shailaja Hittalmani**,** 2011, Response of rice (Oryza sativa L.) genotypes under aerobic conditions. Electronic J. Pl. Breed., **2**(2):194-199.*
10. *Krishna T.V. and Shailaja Hittalmani, 2009, Genetic assessment of root morphology under well water and low moisture stress condition at Reproductive stage. Bull. Biol. Sci. 7 (3), 179-188.*
11. *Lafitte, R., Blum, A. and Atlin, G., 2003, Using secondary traits to help identify drought- tolerant genotypes. In: Fischer, K.S., Lafitte, R., Fukai, S., Atlin, G. and Hardy, B. (Ed.) Breeding rice for drought- prone environments. pp.37-48.*
12. *Lanceras, J.C., Pantuwan, G., Jongdee, B. and Toojinda, T., 2004, Quantitative Trait Loci associated with drought tolerance at reproductive stage in rice. Pl. Physiol. 135: 384-399.*
13. *Malatrasi, M., Close, T.J., and Marmiroli. N., 2002, Identification and mapping of a putative stress response regulator gene in barley. Pl. Mol. Biol., 50(1):143-52.*
14. *Naresh Babu, N, Hittalmani, S**,** Shivakumar, N., Nandini, C., 2011, Effect of drought on yield potential and drought susceptibility index of promising aerobic rice (Oryza sativa L.) genotypes. Electronic Journal of Plant Breeding, **2**(3):295- 302.*
15. *O'Toole, J.C. and Bland, W.L., 1987, Genotypic variation in crop plant root systems. Adv. Agron., **41**: 91-145.*
16. *Oztur, Z.N., Talame, V., Deyholos, M., Michalowski, C.B., Galbraith, D.W., Gozukirmizi, N., Tuberosa, R. and Bohnert. H.J., 2002, Monitoring large-scale changes in transcript abundance in drought and salt stressed barley. Pl. Mol. Biol., 48(5-6):551-73.*

17. *Price, A.H. and Tomos, A.D., 1997, Genetic dissection of root growth in rice (Oryza sativa L.). II. Mapping quantitative trait loci using molecular markers. Theor. Appl. Genet., **95**: 143-152.*

18. *Rabbani, M.A., Maruyama, K., Abe, H., Khan, M.A., Katsura, K., Ito, Y., Yoshiwara, K., Seki, M., Shinozaki, K. and Yamaguchi-Shinozaki, K., 2003, Monitoring expression profiles of rice genes under cold, drought, and high salinity stresses and abscisic acid application using cDNA microarray and RNA-gel blot analyses. Pl. Physiol., 133: 1755-1767.*

19. *Shen, L., Courtois, B., Mcnally, K.L., Robin, S., Li, Z., 2001, Evaluation of near-isogenic lines of rice introgressed with QTLs for root depth through marker-aided selection. Theor. Appl. Genet., **103**: 75-83.*

20. *Thanh, N.O., Zheng, H.G., Dong, N.V., Trinh, L.N., Ali, M.L. and Nguyen, H.T., 1999, Genetic variation in root morphology and microsatellite DNA loci in upland rice (Oryza sativa L.) from Vietnam. Euphytica, **105**: 43-51.*

21. *Vaishali, M.G., Hanamareddy, B., Mane, S., Gireesha, T.M., Shashidhar, H.E. and Hittalmani, S., 2003, Graphical genotyping using DNA markers andevaluation of QTL-NILs of IR64 for root morphological traits, disease resistance and yield traits in rice. Plant and Animal Genome XI Conference, San Diego, USA, p335.*

22. *Venuprasad, R., Bool, M.E., Quiatchon, L and Atlin, G., 2012., A QTL for rice grain yield in aerobic environments with large effects in three genetic backgrounds. Theor. Appl. Genet., 124: 323-332.*

23. *Wang, X., Zhu, J., Mansueto, L. and Bruskiewich, R., 2005, Identification of candidate genes for drought stress tolerance in rice by the integration of a genetic (QTL) map with the rice genome physical map. J. Zhejiang Univ. Sci., 6B (5): 382 –388.*

24. ***www.gramene.org***

25. ***www.ncbi.nlm.nih.gov***

26. ***www.tigr.org***

27. *Yadav, R., Courtois, B., Huang, N. and McLaren, G., 1997, Mapping genes controlling root morphology and root distribution in a doubled haploid population of rice. Theor. Appl. Genet., **94**: 619 –632.*

28. *Yoshida, S. and Hasegawa, S., 1982, The rice root system: Its development and function In : Drought resistance in cereals crops with emphasis on rice, IRRI, Philippines : 53-68.*

APPENDICES

Table 1: Genes Annotated and their Probable Functions in Abiotic and Biotic Stress Response

Sl No.	Gene/Protien expressed	Probable Function
1	Histone H2B	Structural protein
2	**Alpha,alpha-trehalose-phosphate synthase**	Osmoregulation-regulatory
3	Elongation factor 2 (EF-2)	Protein synthesis
4	Aspartate aminotransferase, cytoplasmic isozyme 2	Amino acid metabolism, krebs cycle
5	**Subtilisin-like protease (fragment)**	Stress resistance
6	Beta-6 tubulin	Structural protein
7	Multicatalytic endopeptidase complex chain C8	Structural protein
8	Ribosomal protein L30	Structural protein
9	ADP-ribosylation factor	Vescicle biosynthesis regulation
10	40S ribosomal protein S23 (S12)	Structural protein
11	**Heat shock protein 70 (fragment)**	Stress resistance
12	(S)-tetrahydroberberine oxidase	Alkaloid biosynthesis
13	General negative regulator of transcription subunit 1	Transcription regulation
14	**Fructose-bisphosphate aldolase ,cytosolic**	Stress response
15	Serine palmitoyltransferase 2	Lipid metabolism

Table 1: Contd.,

16	Cysteine synthase ; cytacs1	Salt and metal stress response
17	Putative vacuolar protein sorting-associated protein	Sorting membrane associated proteins
18	Ribosomal protein S10	Structural protein
19	**14-3-3-like protein**	Stress response
20	Glyceraldehyde-3-phosphate dehydrogenase	Carbon metabolism, induction of apoptosis
21	RNA-binding protein 1	Post-transcriptional regulation
22	**Extensin-like protein**	Cell wall morphogenesis regulation
23	Polyribonucleotide nucleotidyltransferase	mrna degradation
24	Ypt family	Vescicular and membrane transport
25	**Gamma-Tip protein**	Osmotic water permeability
26	**Dna j protein homolog 1 (fragment)**	Stress response
27	Enolase 2	Glycolysis
28	**Thioredoxin reductase (NADPH)**	Electron donor, oxidative stress response
29	Histone H2A.IV - Volvox carteri	Structural protein
30	**Probable glutathione S-transferase**	Heat shock stress response
31	Histone H4	Structural protein
32	**Calcium-dependent protein kinase**	Oxidative stress response
33	Ubiquitin	Protein tagging and sorting
34	Ubiquitin / ribosomal protein CEP52	Ribosomal protein
35	Ribosomal protein L32	Structural protein
36	**POS18 protein**	Stress response